WHAT IS CLAIMED IS:

1. A method for determining the presence in a complex protein mixture of at least one target protein in its active state employing a probe composition comprising at least one labeled probe including a ligand and detectable in a liquid chromatography or electrophoretic separation, wherein each of said probes in said composition has specificity for one or a related family of proteins, said method comprising:

conjugating a first sample complex protein mixture with a first probe composition, whereby conjugates of said probes and said target proteins are produced;

sequestering said conjugates with a solid support to which is bound receptor for said ligand;

proteolytically digesting said conjugates either prior or subsequent to said sequestering to produce fragment conjugates;

releasing said fragment conjugates; and

separating said fragment conjugates by at least one of liquid chromatography, electrophoretic separation or mass spectrometry based on the different migration rates of said fragment conjugates, using said label to detect said fragment conjugates, or for mass spectrometry based on ion peaks,

whereby the elution or migration rate of said fragment conjugates indicates the presence of said target protein and/or for mass spectrometry the ion peaks indicate the presence of said target protein.

2. A method according to Claim 1, wherein said separating is with liquid chromatography to produce eluate fractions and comprising the additional step of analyzing said fractions using mass spectrometry.

3. A method according to Claim 2, wherein said mass spectrometry comprises matrix-assisted laser desorption ionization ("MALDI") or electrospray ionization.

4. A method according to Claim 2, wherein said mass spectrometry is MS^n.

5. A method according to Claim 4, wherein said receptor is antibody directed against a fluorescent moiety.

6. A method for determining the presence in a complex protein mixture of at least one target protein in its active state employing a probe composition comprising at least one labeled probe including a ligand and detectable in a liquid chromatography or electrophoretic separation, wherein each of said probes in said composition has specificity for one or a related family of proteins, said method comprising:

conjugating a first sample complex protein mixture with a first probe composition and a second control complex protein mixture with a second probe composition, where said first and second probe compositions differ in having independently detectable labels to produce conjugates of said probes and said target proteins in said complex protein mixtures;

separating said conjugates in said first and second complex protein mixtures by binding said conjugates to a solid support by means of a receptor for said ligand to provide bound conjugates;

washing said bound conjugates free of non-specifically bound components of said complex protein mixtures, followed by releasing said conjugates;

proteolytically digesting proteins at a time prior to releasing;

enriching released conjugate components by separating conjugates by at least one of liquid chromatography and electrophoresis with detection of conjugates by means of said detectable probe;

characterizing said conjugates by their migration rates in said enriching step.

7. A method according to Claim 6, including the additional step of further analyzing said conjugates by mass spectrometry for identification and quantification.

8. A method according to Claim 6, including the additional steps of:

eluting from said liquid chromatography individual fractions in wells;

screening said wells for the presence of said detectable probe; and

transferring fractions containing said detectable probe for analyzing by said mass spectrometry.

9. A method according to Claim 6, wherein said label is a fluorescent label and said separating is with antibodies to said fluorescent label.

10. A method according to Claim 9, wherein said fluorescent labels in said first and second probe compositions are matched to similarly affect the migration of said conjugates.

11. A method according to Claim 6, wherein said proteolytically digesting is with two different proteases.

12. A method according to Claim 6, wherein said second complex protein mixture differs from said first complex protein mixture in being denatured by a non-covalent denaturing agent.

13. A method according to Claim 6, wherein the amount of said conjugate is determined by the relative amounts of said detectable probes in said conjugates of the same target protein from said first and second complex protein mixtures.

14. A method for determining the presence in a complex protein mixture of at least one target protein in its active state employing a probe composition comprising at least one isotopically labeled probe comprising a label detectable in a liquid chromatography or electrophoretic separation, wherein each of said probes in said composition has specificity for one or a related family of proteins, said method comprising:

conjugating a first sample complex protein mixture with a first probe composition and a second control complex protein mixture with a second probe composition, where said first and second probe compositions differ in their isotopic labeling to produce conjugates of said probes and said target proteins;

enriching conjugate components by separating conjugates by at least one of liquid chromatography and electrophoresis with detection of conjugates by means of said detectable label to provide separately enriched fractions;

analyzing said separately enriched fractions by mass spectrometry and obtaining a comparison of the amount of conjugates from each of said first and second complex protein mixtures;

whereby the mass spectrometry provides identification and quantitation of said target proteins.

15. A method according to Claim 14, wherein said mass spectrometry is MALDI and including the steps of:

introducing fractions into a MALDI plate;

detecting enriched fractions containing conjugates by means of said detectable label.

16. A method according to Claim 9, wherein said mass spectrometry is ESI.

17. A method for determining the presence in a complex protein mixture of at least one target protein in its active state employing a probe composition comprising at least one labeled probe including a ligand and detectable in a liquid chromatography or electrophoretic separation, wherein each of said probes in said composition has specificity for one or a related family of proteins, said method comprising:

conjugating a first sample complex protein mixture with a first probe composition, whereby conjugates of said probes and said target proteins are produced;

combining said conjugates with a at least one protease bound to a solid surface and incubating for sufficient time for fragment conjugates to be formed;

sequestering said conjugates with a solid support to which is bound receptor for said ligand;

proteolytically digesting said conjugates either prior or subsequent to said sequestering to produce fragment conjugates;

releasing said fragment conjugates; and

separating said fragment conjugates by at least one of liquid chromatography or electrophoretic separation based on the different migration rates of said fragment conjugates, using said label to detect said fragment conjugates,

whereby the elution or migration rate of said fragment conjugates indicates the presence of said target protein.

18. A method according to Claim 17, wherein fragment conjugates from fractions of said liquid chromatography are analyzed by mass spectrometry.

19. A method according to Claim 17, wherein fragment conjugates from said capillary electrophoresis are analyzed by mass spectrometry.

20. A kit comprising a plurality of affinity based labeled probes comprising a ligand, at least one protease, and a protein standard.

21. A method for determining the presence, amount, or activity of one or more active target proteins in a complex protein mixture, the method comprising:

(a) contacting said complex protein mixture with an activity based probe that specifically binds to one or more active target proteins;

(b) proteolyzing said active target protein(s) to produce a product mixture;

(c) separating said product mixture into one or more components, one or more of which comprise peptides bound to said probe; and

(d) generating a signal from said peptides bound to said probe, wherein said signal is correlated to the presence, amount, or activity of said one or more active target proteins in said complex protein mixture.

22. A method according to claim 21, wherein said separating step (c) comprises sequestering one or more peptides bound to said probe using a receptor that specifically binds to said probe.

23. A method according to claim 22, wherein said probe comprises a fluorescent moiety, and said receptor is an antibody or fragment thereof that binds to said fluorescent moiety.

24. A method according to claim 21, wherein said probe comprises a fluorescent moiety, and said signal is a fluorescent signal generated from said probe.

25. A method according to claim 21, wherein said signal is a mass spectrum.

26. A method according to claim 21, wherein, prior to said proteolyzing step (b), one or more components of said complex protein mixture not bound to said probe are removed from said complex protein mixture.

27. A method according to claim 21, wherein said probe comprises a label selected from the group consisting of a fluorescent moiety and an isotopic label.

28. A method according to claim 21, wherein said separating step (c) comprises one or more separation methods selected from the group consisting of affinity separation, gel electrophoresis, capillary electrophoresis, liquid chromatography, HPLC, electrospray ionization and MALDI.

29. A method according to claim 21, wherein prior to said proteolyzing step (b), said one or more active target proteins bound to said probe are bound to a solid support.

30. A method according to claim 21, wherein said method further comprises adding one or more standard proteins to said complex protein mixture prior to said proteolysis step (b).

31. A method according to claim 30, wherein said standard protein(s) are labeled with an activity based probe prior to addition to said complex protein mixture.

32. A method according to claim 31, wherein said standard protein(s) are labeled with an activity based probe comprising a fluorescent moiety that is distinguishable from said activity based probe contacted with complex protein mixture.

33. A method for comparing the presence, amount, or activity of one or more active target proteins in two complex protein mixtures, the method comprising:

(a) contacting a first complex protein mixture with a first activity based probe, and contacting a second complex protein mixture with a second activity based probe, wherein each of said first and second probes specifically bind to one or more active target proteins in each complex protein mixture;

(b) combining said first and said second complex protein mixtures to form a combined complex protein mixture;

(c) proteolyzing said active target protein(s) in said combined complex protein mixture to produce a product mixture;

(d) separating said product mixture into one or more components, one or more of which components comprise peptides bound to said first probe and peptides bound to said second probe; and

(e) comparing a first signal generated from said peptides bound to said first probe, and a second signal generated from said peptides bound to said second probe, wherein said comparison is correlated to the relative presence, amount, or activity of said one or more active target proteins in said first and second complex protein mixtures.

34. A method according to claim 33, wherein said separating step (d) comprises sequestering one or more peptides bound to said first probe using a first receptor that specifically binds to said first probe and sequestering one or more peptides bound to said second probe using a second receptor that specifically binds to said second probe.

35. A method according to claim 34, wherein said first and second probes comprise a fluorescent moiety, and said receptors are antibodies or fragments thereof that bind to said fluorescent moiety.

36. A method according to claim 34, wherein said first and second probes each comprise a fluorescent moiety, said first and second signals are fluorescent signals generated from said first and second probes, and said first and second signals are distinguishable from one another.

37. A method according to claim 33, wherein said first and second probes are isotopically different, said first and second signals are mass spectra, and said first and second signals are distinguishable from one another.

38. A method according to claim 33, wherein, prior to said proteolyzing step (b), one or more components of said combined complex protein mixture not bound to said first or second probes are removed from said complex protein mixture.

39. A method according to claim 33, wherein said first and second probes each comprise a label selected from the group consisting of a fluorescent label and an isotopic label.

40. A method according to claim 33, wherein said separating step (d) comprises one or more separation methods selected from the group consisting of affinity separation, gel electrophoresis, capillary electrophoresis, liquid chromatography, HPLC, electrospray ionization, and MALDI.

41. A method according to claim 33, wherein prior to said proteolyzing step (b), said one or more active target proteins bound to said first and second probes are bound to a solid support.

42. A method according to claim 33, wherein said method further comprises adding one or more standard proteins to said first, second, or combined complex protein mixture prior to said proteolysis step (b).

43. A method according to claim 42, wherein said standard protein(s) are labeled with an activity based probe prior to addition to said first, second, or combined complex protein mixture.

44. A method according to claim 43, wherein said standard protein(s) are labeled with an activity based probe comprising a fluorescent moiety that is distinguishable from said activity based probe contacted with complex protein mixtures.

45. A method of correlating a separation profile to a peptide having a known sequence, wherein said peptide is bound to an activity based probe, the method comprising:
generating said separation profile for said peptide by performing one or more separation methods and generating a signal from said peptide bound to said probe, wherein said separation profile is characteristic of said peptide having said known sequence.

46. A method according to claim 45, wherein said method further comprises obtaining the known sequence of said peptide by mass spectrometry.

47. A method according to claim 46, wherein said method further comprises:

(a) contacting a protein sample comprising an amino acid sequence encoding said peptide with an activity based probe that specifically binds to said amino acid sequence;

(b) proteolyzing said protein sample to produce a product mixture;

(c) separating peptides bound to said probe from said product mixture;

(d) generating a mass spectrum from said peptides bound to said probe, wherein said mass spectrum provides the known sequence of said peptide; and

(e) generating said separation profile by capillary electrophoresis of said peptides bound to said probe, wherein said separation profile is one or more migration time(s) of said peptides bound to said probe.

48. A method according to any one of claims 1, 6, 14, 17, 21, or 33, wherein said complex protein mixture is a proteome.